Selective Enrichment of Peptides by Utilizing Dispersive Pipette Extraction on Automated Liquid Handler for High Throughput Discovery and Processing

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ABSTRACT

According to a database established from human hemofiltrate, approximately 5000 different peptides were recorded. Over 95% of the detected masses were smaller than 15,000 Da and a small percentage (7%) of the detected masses represented sequences from peptide hormones, growth factors and cytokines. These low molecular weight (LMW) peptides/proteins are attractive targets for monitoring human health. Here, we demonstrate a proof of concept size exclusion chromatography technique using INtip, dispersive pipette extraction chemistries (IMCStips™) for rapid isolation of LMW peptides(Bradykinin and Angiotensin I) by using various pore sizes ranging from 30 Å to 60 Å.

INTRODUCTION

Solid phase extraction relies on physicochemical properties in order to partition selected analytes from sample solutions. Majority, if not all SPE products are limited in its capability for purifying and enriching peptides (1-10 kDa) from large pool of interfering proteins (>10 kDa) or small molecules (< 1 kDa). In this study, IMCStips are used to selectively enrich for peptides from biofluids on a 96-channel liquid handler, Microlab® NIMBUS® from Hamilton Company. Mulitvariant factors are imposed in high throughput manner to optimize the isolation conditions, and thereafter the high throughput for selected peptides is achieved. Transferring the sample preparation process with IMCStips required no additional equipment to the automated or semi-automated liquid handling system.

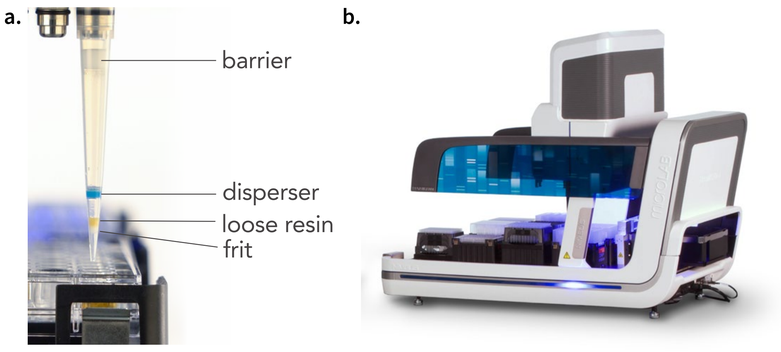

Figure 1. (a) IMCStips diagram (b) Nimbus automated liquid handler

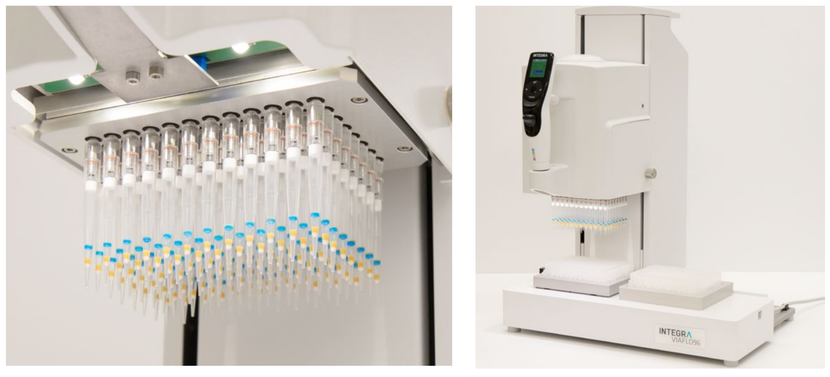

Figure 2. IMCStips on Integra VIAFLO96

MATERIALS AND METHODS

Peptide recovery with IMCStips was assessed using various peptides spiked in the biofluid. All solvents were MS grade and purchased from Fisher Scientific. IMCStips were developed by Integrated Micro-Chromatography Systems, LLC (Irmo, SC). All tips were 300 µL volume pipette tips for Nimbus or Integra ViaFlo96. The IMCStips contained 5 mg of silica particles from various suppliers with different porosities. For peptide extraction process optimization, two-level factorial design was implemented to correlate the interaction between pH and ionic strength or pH and solvent.

For proof of concept experiments, the matrix consisted of 10 µg of partially trypsin digested BSA and α-casein and 100 ng of four peptides (bradykinin, angiotensin I and II, leptin) were spiked into the matrix. The samples were then extracted using IMCStips with 5 mg of silica using the following steps. The samples were then extracted using IMCStips with 5 mg of silica using the following steps.

1. Condition (0.5% formic acid in acetonitrile, 200 µL, 3 aspiration/dispense cycles)
2. Equilibration (5% acetonitrile, 0.5% formic acid in water, 150 µL, 3 aspiration/dispense cycles)
3. Sample/peptide enrichment step (150 µL, 30 aspiration/dispense cycles with 30 second hold) Wash 1 (5% acetonitrile, 0.5% formic acid in water, 150 µL, 3 aspiration/dispense cycles)
4. Wash 2 (10% acetonitrile, 0.5% formic acid in water, 150 µL, 3 aspiration/dispense cycles)
5. Elution (50% acetonitrile, 0.5% formic acid, 200 µL, 5 aspiration/dispense cycles

Dry down elution and reconstitute sample in 100 µL of 5% acetonitrile, 0.5% formic acid in water. 5 µL was injected and separated on C18 Hypersil Gold column.

RESULTS

Untargeted peptide enrichment process is still a work in progress and requires extensive investment of time and resources. The method described here provides one of the experimental strategies in achieving high-throughput enrichment of peptides for downstream biomarker discovery studies.

Table 1. Amino acid sequences of polypeptides

Name	Amino Acid Sequence	Theoretical MW	Calculated pI
Bradykinin	RPPGFSPFR	1060.23	12
Angiotensin I	DRVYIHPFHLVI	1507.83	6.92
Angiotensin II	DRVYIHPFHL	1295.68	6.92
Leptin peptide (93-105)	NVIQISNDLENLR	1526.81	4.37
Hirudin (55-65)	DFEEIPEEYLQ	1411.5	3.45
Calcitonic(15-29)	DFNKFHTFPQTAIGV	1721.9	6.74
Erythropoietin(EPO, 117-131)	EAISPPDAASAAPLR	1465.6	4.37
MUC5AC 3	GTTPSPVPTTSTTSAP	1501.6	5.52
Carcinoembryonic Antigen	TYLWWVNNQSL	1423.6	5.18

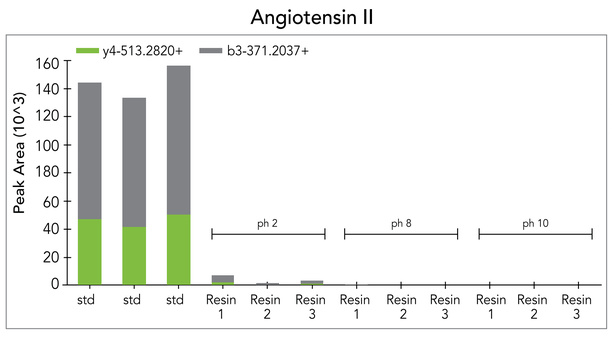

Figure 3. Detection of human angiotensin II peptide in peptide/protein mixture. 10 pmol angiotensin II was spiked in 10 ug of BSA/casein. The recoveries were measured using three different pH conditions with three different resin types.

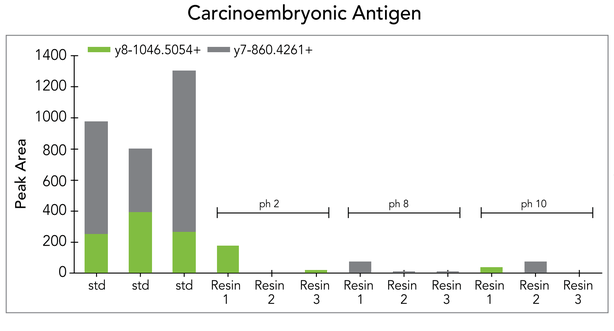

Figure 4. Detection of carcinoembryonic antigen (CEA) peptide fragment in peptide/protein mixture. 10 pmol of CEA peptide fragment was spiked in 10 ug of mixture. The recoveries were measured using three different pH conditions and three different resin types.

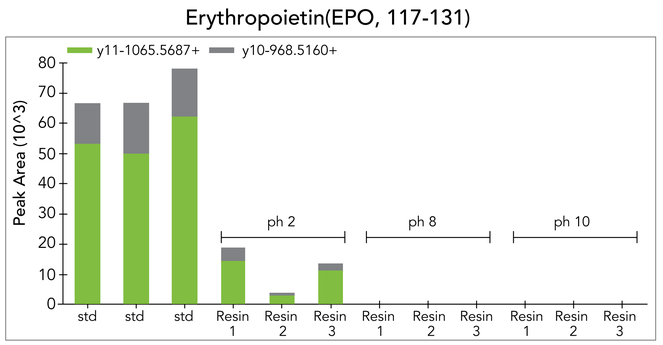

Figure 5. Erythropoietin peptide fragment recoveries from partial digests of BSA/alpha casein in various pH conditions using silica particles with different porosities. 10 pmol of CEA peptide fragment was spiked in 10 ug of mixture.

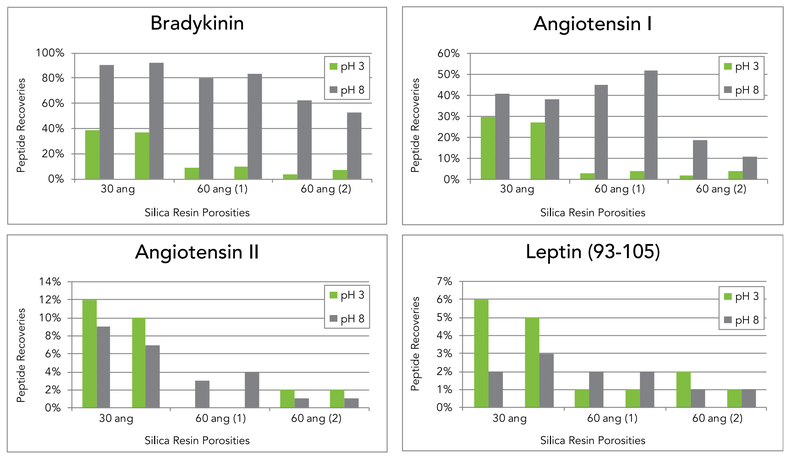

Figure 6. Various peptide recoveries from partial digests of BSA/alpha casein in acidic or basic conditions using various silica particles with different porosities.

CONCLUSIONS

- Porous silica (30 Å) packaged in dispersive pipette extraction demonstrated efficient enrichment of Bradykinin
- Larger pore (60 Å) silica particles, despite larger surface area showed lower recoveries
- Surface chemistries / pH / pI impact recoveries of peptides

Future Direction

- Increase complexity of the sample matrix (biological fluids)
- Modify the surface chemistries of the resin while retaining small porosities to expand size exclusion toolkit

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